ABSTRACT

PURPOSE: To investigate the reproducibility of the experimental model of face allotransplantation in rats in Brazil.

METHODS: Eighteen rats were operated, nine-nine donors recipients. Animals underwent transplantation of the left hemiface, with periorbital and scalp. Transplants were made from donor Wistar rats to recipients Lewis rats. Flaps were based on the common carotid artery and the external jugular vein of the donor animal and the anastomosis in the recipient area was performed in common carotid artery (end-to-side) and in external jugular vein (end-to-end).

RESULTS: Of the nine recipient animals operated, six survived and three progressed to death in the first days after surgery (survival rate = 67%). The mean time of the procedure was 252 minutes and the mean time of flap ischemia was 95 minutes. The five surviving animals were sacrificed at 14 days, in good general condition and without signs of tissue rejection.

CONCLUSIONS: The experimental model of face allotransplantation in rats is reproducible in our midst. Duration of surgery, time of flap ischemia, animal survival rate and complications observed were similar to those described in the literature.

Key words: Models, Animal. Facial Transplantation. Rats.
Introduction

Some facial defects are complex, with large proportions, and may occur due to traumatic injuries, severe burns or extensive oncologic resections, leading to an extensive tissue loss involving skin, soft tissue (subcutaneous tissue, muscles, vessels and nerves), osteocartilaginous structures and facial functional subunits (nose, lips and eyelids). This leads to three-dimensional defects with a difficult reconstruction, that require multiple surgical procedures. Reconstructive surgery of the face aims to restore anatomical normality to obtain satisfactory aesthetic and functional results.

Recently, a new approach to the treatment of complex facial defects has been proposed: the face transplantation. It consists of an allogenic flap formed by heterogeneous tissues of the face such as skin, muscle, bone and other supplied by a pedicle. This procedure has become a viable option, with many successful cases around the world. The applicability of composite tissue allotransplantation has been made possible by the establishment of microsurgical techniques, the recent advancement of immunosuppression regimes, by anatomical cadaver studies and development of experimental animal models.

Most research on face transplants have been performed in experimental models, which use various animals, especially rats. In 2003, it was described the first experimental model of face transplant in rats (the face of the donor animal to the face of the recipient animal). Since then, several other models have been proposed, with emphasis on the use of only a hemiface in order to simplify the procedure and make it faster, without prejudice to studies of immunosuppressive and tolerance induction.

The aim of this study was to test the reproducibility of the experimental model of face allotransplantation in rats in Brazil.

Methods

Study conducted at the Plastic Surgery and Microsurgery Laboratory, Division of Plastic Surgery, Faculty of Medicine, University of Sao Paulo, Brazil. All animals were treated strictly following the principles of Brazilian laws 9.605/98 and 11.794/08, the Ethical Principles in Animal Experimentation of CONCEA (National Council for the Control of Animal Experimentation) and the Principles for Research Involving Animals (Geneva, 1985). This study received previous approval of the local Ethics Committee (302/10).

Eighteen rats were operated (weight 300-350g), nine-nine donors recipients. Anesthesia was induced with intraperitoneal pentobarbital sodium and dissections and vascular anastomoses were performed with an optical microscope Zeiss x40 magnification, using mononylon 10-0. After the procedure, receptors rats remained in the vivarium and received analgesia with buprenorphine in the first two days.

Animals underwent transplantation of the left hemiface, with periorbital and scalp. The transplants were made from donor Wistar rats to recipients Lewis rats. Flaps were based on the common carotid artery and the external jugular vein of the donor animal and the anastomosis in the recipient area was performed in common carotid artery (end-to-side) and in external jugular vein (end-to-end).

Immunosuppression was adopted to prevent acute rejection, using cyclosporin A (16 mg/kg/day) for the first 14 days, when the animals were sacrificed by lethal intraperitoneal dose of pentobarbital (100 mg/kg).

Statistical analysis was performed using Microsoft Excel for Mac 2011 version 14.3.9.

Surgical technique for donor animal

First, a left cervical incision 1.0 cm above the sternum was made, later continuing 1.0 cm caudal to the ear level, another elliptical incision was made around the eyes and the last taken 2.0 cm caudal to the tip of the nose continuing perorally 1.0 cm above the commissure and the lower lip (Figure 1). With the animal in supine position, the external jugular vein and its branches were identified, and the glossopharyngeal nerve was removed to expose the common carotid artery and its branches.
After that, with the animal in lateral position, the posterior auricular artery and vein were dissected and the ear was included in the flap with the release of its external cartilaginous canal. Then, with the animal in the prone position, the flap was dissected in the subgaleal plane and around the periorbital and perioronasal incision, being connected to the internal maxillary artery.

The flap was then completely released from all adjacent tissues. After ligature of the internal carotid artery and cervical branches of the external carotid artery, common carotid artery and jugular vein were separated, creating the vascular pedicle of the donor flap (Figures 2 and 3).

**Results**

Of the nine recipient animals operated, six survived and three progressed to death in the first days after surgery, with an animal survival rate of 67% (Table 1). Most complications were observed in the first four days after the procedure, as partial or total flap necrosis and poor clinical status. Two animals evolved with a little partial flap necrosis, with good recovery, surviving until they were sacrificed.

**TABLE 1 - Data of the nine operated animals.**

<table>
<thead>
<tr>
<th>Rat</th>
<th>Recipient Surgery (minutes)</th>
<th>Flap Ischemia (minutes)</th>
<th>Survival (days)</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>285</td>
<td>120</td>
<td>1</td>
<td>death</td>
</tr>
<tr>
<td>2</td>
<td>260</td>
<td>105</td>
<td>3</td>
<td>total flap necrosis and death</td>
</tr>
<tr>
<td>3</td>
<td>245</td>
<td>95</td>
<td>14</td>
<td>partial flap necrosis</td>
</tr>
<tr>
<td>4</td>
<td>270</td>
<td>110</td>
<td>14</td>
<td>none</td>
</tr>
<tr>
<td>5</td>
<td>225</td>
<td>80</td>
<td>14</td>
<td>none</td>
</tr>
<tr>
<td>6</td>
<td>250</td>
<td>90</td>
<td>14</td>
<td>partial flap necrosis</td>
</tr>
<tr>
<td>7</td>
<td>215</td>
<td>85</td>
<td>4</td>
<td>poor clinical status and death</td>
</tr>
<tr>
<td>8</td>
<td>260</td>
<td>80</td>
<td>14</td>
<td>none</td>
</tr>
<tr>
<td>9</td>
<td>255</td>
<td>95</td>
<td>14</td>
<td>none</td>
</tr>
</tbody>
</table>

The mean time of the recipient transplantation procedure was 252 minutes and the mean time of flap ischemia was 95 minutes (Table 2). The six surviving animals were sacrificed at 14 days, in good general condition and without signs of tissue rejection (Figures 4 and 5).

**TABLE 2 - Statistical analysis of data.**

<table>
<thead>
<tr>
<th></th>
<th>Recipient Surgery (minutes)</th>
<th>Flap Ischemia (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>251.66</td>
<td>95.55</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>21.50</td>
<td>13.79</td>
</tr>
<tr>
<td>Median</td>
<td>255</td>
<td>95</td>
</tr>
</tbody>
</table>

**Surgical technique for recipient animal**

The same donor animal incisions were made on the recipient rat to create a defect on the face and scalp. The periorbital region and perioronasal tissues were preserved. The external jugular vein was identified as the recipient vein and prepared for end-to-end anastomosis as well as the common carotid artery was identified as the recipient artery and prepared for end-to-side anastomosis.

**FIGURE 2** - Outside hemifacial flap.

**FIGURE 3** - Inside hemifacial flap, with artery and vein holded by forceps.

**FIGURE 4** - Anterior view. Postoperative, with flap in good condition.
Discussion

Most research on face transplants have been performed in experimental models, which use various animals such as rats, pigs, dogs and primates. These models are suitable because the facial circulation of these animals is provided mainly by branches of the external carotid artery and jugular vein, as in humans. Among them, rats are commonly used.

Many of these models have been described and developed in recent years, mostly by Siemionow et al., in the United States. The development of an experimental model requires a certain learning curve and is followed by high animal mortality due to the complexity of this procedure. The use of experimental models of allotransplants has enabled the development of surgical techniques and immunosuppressive protocols, contributing to the future adoption of complex facial defects.

In 2003, the first experimental model of face transplantation in rats was described by Siemionow et al., as an orthotopic facial allotransplant. The donor rat was of Lewis Brown Norway strain (dark hair) and the transplanted segment included the periorbital, malar, forehead and scalp, based on the common carotid artery and external jugular vein. The recipient rat was of Lewis strain (light hair) and the flap vessels anastomosis were performed in the external carotid artery and the facial vein. The difference in color in the coat of these animals aimed to show the contrast of dark hair in the donor rat with a light coat of the recipient rat.

Since then, several other models have been proposed, with inclusion of facial segments as mandible, maxilla, tongue, etc. Among these, we highlight the use of only rat hemiface instead of full face, in order to simplify the procedure and to make it faster, without prejudice to the studies of immunosuppressive and tolerance induction. As the facial transplant is a very complex procedure and have a high animal mortality rate, other studies have correlated the sites of vascular anastomoses with the rat survival rate, pointing out the sites that have been most favorable.

In 2004, Demir et al. proposed transplanting only the hemiface mouse. In eighteen transplanted animals, the mean operating time was 180 minutes and the mean time of flap ischemia was 80 minutes, achieving survival rates of 100% of the animals, and no major complications in the postoperative period. In 2006, Yazici et al. described the transplantation of hemiface with rat calvaria, and the mean time of flap ischemia was 60 minutes, and all the seven operated animals survived. These two articles came from the group headed by Siemionow, in Cleveland, United States. They are pioneer in experimental models of face transplants, and they have a great experience and a long learning curve.

In 2012, Sucher et al. developed a novel surgical technique with which to perform hemiface transplantation in mice. The surgical procedure was performed with a success rate of 78% and the mean operating time was 150 minutes for the recipient animal.

In 2013, Climov et al. studied the learning curve of hemifacial transplantation in rats by comparison between two operators: medical student trained in basic microsurgery (10 rats) and an experienced microsurgeon (five rats). Transplantation procedure duration mean time (donor plus recipient) was 930 minutes to the microsurgeon and 470 minutes to the medical student. Flap ischemia mean time was 90 minutes to the microsurgeon and 133 minutes to the medical student. The global survival rate was 73% of 15 animals.

In our study, we only performed transplantation of rat hemiface, because it is technically simpler and it is associated with lower mortality rates. Except by the Cleveland group, which has a long learning curve and very good outcomes, our results were similar to those described in the literature, such as duration of surgery (252 minutes), time of flap ischemia (95 minutes), survival rate (66%) and complications observed.

As future perspectives, this experimental model can serve as a basis for establishing experimental protocols of vascularized composite facial allotransplantation tissues. Thus, we intend to collaborate with the performing of a human face transplant in Brazil.

Conclusions

The experimental model of face allotransplantation in rats is reproducible in our midst. Duration of surgery, time of flap ischemia, animal survival rate and complications observed were similar to those described in the literature.
References


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